USARIEM TECHNICAL REPORT T-01/14

SKIN EXPOSURE REDUCTION PASTE AGAINST CHEMICAL WARFARE AGENTS (SERPACWA) - EFFECT OF ALCOHOL PRE-TREATMENT

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June 2001

U.S. Army Research Institute of Environmental Medicine
Natick, MA 01760-5007

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	June 2001	Technical Report

4. TITLE AND SUBTITLE

SKIN EXPOSURE REDUCTION PASTE AGAINST CHEMICAL WARFARE AGENTS - EFFECT OF ALCOHOL PRE-TREATMENT

5. FUNDING NUMBERS

6. AUTHOR(S)
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

U.S. Army Research Institute of Environmental Medicnie
42 Kansas Street
Natick, MA 01760-50078

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

Skin Exposure Reduction Paste Against Chemical Warfare Agents (SERPACWA, previously known as Topical Skin Protectant), has been proposed to complement chemical protective clothing. The skin surface at the closure sites of chemical protective clothing and gear may be vulnerable to chemical warfare agent exposure. The use of SERPACWA on the skin at these closure sites may provide protection against percutaneous threat of chemical agents. The Food and Drug Administration (FDA) has approved the New Drug Application (NDA #21-084) as a safe and effective topical drug for use by soldiers for protection against chemical warfare agents. However, the FDA and the Army Combat Developers requested additional studies to determine the optimal conditions for SERPACWA's use. In this paper we report the outcome of one experiment, the purpose of which was to determine if the use of isopropyl alcohol to clean the skin prior to application of SERPACWA had any effect on how well SERPACWA served as a protective barrier. Paired test sites on the volar surfaces of the forearms of six volunteer soldiers either were or were not pre-treated with alcohol and were or were not treated with SERPACWA. All four sites were challenged with methyl nicotinate (Mnic). Mnic exposure results in redness and sometimes swelling from vasodilation caused by the skin's non-immunologic contact reaction to this chemical. Timed skin responses to Mnic were visually evaluated and were measured as flux calculations from laser-Doppler imagery (LDI). When SERPACWA protected sites were evaluated following Mnic challenge, comparisons between alcohol pre-treated vs. no alcohol pre-treatment sites were not different (flux = 51 vs. 61, $p>0.05$, and visual scores = 0.1 vs. 0.1, $p>0.05$). When skin sites not protected by SERPACWA were evaluated following Mnic challenge, the flux and visual scores for alcohol pre-treated sites were significantly higher than sites with no alcohol pre-treatment (flux = 291 vs. 185, $p<0.05$, and visual scores = 1.4 vs. 1.1, $p<0.05$). These results indicate that alcohol pre-treatment is not necessary for SERPACWA's effectiveness, and in the absence of SERPACWA, appears to exacerbate the effect of the challenge agent, Mnic.

14. SUBJECT TERMS

topical irritant, skin protection, isopropyl alcohol

15. NUMBER OF PAGES
14

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	

DISCLAIMER STATEMENTS

The views, opinions and/or findings contained in this publication are those of the authors and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

Human subjects participated in this study after giving their free and informed voluntary consent. Investigators adhered to AR 70-25 and USAMRMC Regulation 70-25 on the use of volunteers in research. For the protection of human subjects, the investigators adhered to policies of applicable Federal Law CFR 46.

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ACKNOWLEDGMENTS

The authors would like to especially thank the soldiers who volunteered to participate as test subjects. We also thank the USARIEM soldiers and civilians who served as study technicians: Janet Staab, Scott Robinson, CPT Sam Chevront, Robert Carter, and Lauren Cavanaugh; the statistician: Robert Wallace; and the physicians who provided medical coverage: MAJ Bernard Bettencourt (Medical Monitor) and CPT Patrick Devine (Covering Physician).

Funding for this (Phase IV Testing) study was provided by the U.S. Army Medical Materiel Development Activity.

EXECUTIVE SUMMARY

Skin Exposure Reduction Paste Against Chemical Warfare Agents (SERPACWA), previously known as Topical Skin Protectant (TSP), has been proposed to augment the protection afforded by chemical protective clothing. The skin surface at the closure sites of chemical protective clothing and gear may be vulnerable to chemical warfare agent exposure. The use of SERPACWA on the skin at these closure sites may provide protection against the percutaneous threat of chemical agents. The Food and Drug Administration (FDA) has approved the New Drug Application (NDA #21-084) as a safe and effective topical drug for use by soldiers for protection against chemical warfare agents. However, the FDA and the Army Combat Developers requested additional studies to determine the optimal conditions for SERPACWA use. In this paper we report the outcome of the second of four experiments conducted to determine optimal SERPACWA usage. The purpose of this experiment was to answer this question: Does the use of isopropyl alcohol to clean the skin prior to application of SERPACWA have any effect on how well SERPACWA serves as a protective barrier?. The U.S. Army Research Institute of Environmental Medicine (USARIEM), at the request of the U.S. Army Medical Materiel Development Activity (USAMMDA), conducted a study under Good Clinical Practices (GCP) guidelines with the objective of providing answers to this and several other questions outlined in the study protocol (regarding challenge agent concentration and SERPACWA durability, application timing, and reapplication). Paired test sites on the volar surfaces of the forearms of six volunteer soldiers either were or were not pre-treated with alcohol and were or were not treated with SERPACWA. All four forearm sites were challenged with methyl nicotinate (Mnic). Mnic exposure results in redness and sometimes swelling from vasodilation caused by the skin's non-immunologic contact reaction to this chemical. Timed skin responses to Mnic were visually evaluated and were also measured as flux calculations from laser-Doppler imaging (LDI). When SERPACWA protected sites were evaluated following Mnic challenge, comparisons between alcohol pre-treated vs. no alcohol pre-treatment sites were not different (flux = 51 vs. 61, $p>0.05$, and visual scores = 0.1 vs. 0.1, $p>0.05$). When skin sites not protected by SERPACWA were evaluated following Mnic challenge, the flux and visual scores for alcohol pre-treated sites were significantly higher than sites with no alcohol pre-treatment (flux = 291 vs. 185, $p<0.05$, and visual scores = 1.4 vs. 1.1, $p<0.05$). These results indicate that alcohol pre-treatment is not necessary for SERPACWA's effectiveness, and in the absence of SERPACWA, appears to exacerbate the effect of the challenge agent, Mnic.

INTRODUCTION

Chemical warfare agents (CWA) continue to pose a threat to U.S. warfighters and peacekeepers. Skin Exposure Reduction Paste Against Chemical Warfare Agents (SERPACWA), previously known as Topical Skin Protectant (TSP), has been proposed to compliment the use of chemical protective clothing and gear as additional protection against the percutaneous threat of CWA at the closure sites of these garments (7). It is non-reactive, non-wetting and immiscible with most other chemicals. SERPACWA is a thick cream that can be spread in a thin, even layer on the skin, comprised of a Teflon®-like mixture in perfluoroalkylpolyether which creates an inert, passive physical barrier over the stratum corneum (20, 21). This barrier prevents penetration and percutaneous absorption of a wide variety of substances including chemical or biological warfare agents. It is non-irritating to skin and eyes and can be used over a wide range of temperatures (20, 21, 30). These characteristics make it an ideal candidate for preventing skin contact with CWA.

SERPACWA studies have included the use of a skin challenge agent such as an allergen or toxin. To test SERPACWA's effectiveness, the Army and others have used urushiol (poison ivy) extract and methyl nicotinate (Mnic) as challenge agents in human subject studies (3, 7, 30). These challenge agents cause skin erythema and vesiculation following unprotected skin exposure. SERPACWA treated skin was protected against both of these challenge agents (3, 7). Using Mnic as the challenge agent, SERPACWA was shown to be an effective skin protectant for up to an hour in sweating humans (7). The rapid skin response to Mnic (non-immunologic contact reaction), manifested by erythema or urtication, generally within minutes, makes it useful as a challenge agent.

The FDA reviewed the New Drug Application (NDA) for SERPACWA and approved it as safe and effective for its intended use. However, the FDA and the Combat Developers requested that additional questions regarding SERPACWA use be answered. This report addresses one of those questions: Is cleaning the skin with isopropyl alcohol before SERPACWA application necessary? The additional questions addressed by the same protocol, but not reported on in this paper, concern SERPACWA's effectiveness relative to application timing, length of effectiveness, and effectiveness of re-application. In addition, an experiment was conducted to determine the most effective dose and timing for the challenge agent, Mnic. Those results are reported by Kesick et al. (16).

The purpose of this experiment was to determine if cleansing the skin with isopropyl alcohol prior to SERPACWA application affects SERPACWA's effectiveness in protecting the skin from the Mnic challenge. Isopropyl alcohol was used to clean the challenge sites for the urushiol challenge in one of the two pivotal studies supporting the NDA for SERPACWA (29,30). Therefore, the FDA questioned if this step was necessary to demonstrate SERPACWA's effectiveness. The effects of alcohol alone and in conjunction with Mnic application are also addressed in this report.

METHODS

This research study was conducted in compliance with applicable Good Clinical Practice regulations, with USARIEM Standard Operating Procedures, and as described in the study protocol (USARIEM Protocol #H00-17/HSRRB Log No. A-9925).

STUDY DESIGN

Six volunteers were tested in this experiment. The sample size was estimated by power analysis for a paired t-test using standard deviations derived from LDI flux measurements (96 and 73 for untreated and treated sites, respectively) from a previous SERPACWA study (7). The sample size was estimated using an alpha = 0.05 and power = 0.80.

Two 2.4 cm diameter circular sites were identified on the volar surface of each forearm for each of the six subjects, as shown in Figure 1. One site on each arm was prepped with alcohol prior to SERPACWA application. SERPACWA was applied to the prepped and non-prepped sites on one arm. After 60 min, each site received a 2-min challenge with Mnic. LDI scans were performed prior to the alcohol prep (Scan 1), after SERPACWA application (Scan 2), and approximately 15 min post-Mnic challenge (Scan 3).

Test Subject Selection

Six healthy male soldiers, at the Natick Soldier's Systems Center, on Temporary Duty for the purpose of being available to volunteer for research studies) volunteered to participate in this experiment (identified as Experiment II in the study protocol) after being fully informed of all test procedures and risks, after reading and signing an approved, informed consent document. Following their signed consent, they were cleared by the medical monitor and screened with respect to the inclusion/exclusion criteria (listed below). Their characteristics (mean ± standard deviation) were as follows: Age 20 (±3) years, Height 1.7 (±0.1) m, and Weight 84.5 (±7.5) kg. Five of the volunteers were right-handed, 1 was ambidextrous (wrote left-handed). Four of the volunteers were Caucasian, 1 was African American, and 1 was Hispanic.

Inclusion Criteria – Before acceptance as test subjects, volunteers had to meet the following criteria:

1. Volunteers were active duty military; unrestricted as to race, ethnicity or gender; 18-55 years of age; and generally in good health as established through medical examination.
2. Volunteers had a resting blood pressure no greater than 140/90 and a resting pulse rate of 50-100 bpm.
3. Female volunteers had a negative urine pregnancy test at enrollment.
4. Volunteers were willing to abide by the rules of the study.

5. Volunteers' volar forearm and wrists were free of scars, tattoos, cuts or abrasions that would interfere with test measurements, and the width of the volar surface exceeded 5.5 cm as measured at the wrist.
6. Volunteers signed the informed consent document.
7. Volunteers were willing and able to refrain from alcohol intake for 24 hours prior to the start of each day of testing.
8. Volunteers were willing and able to refrain from using any medications (prescription or over-the-counter) except for oral contraceptives for 2 days prior to testing, until testing was completed. These included drugs classified as antihistamines, anti-inflammatories including corticosteroids, cortisone, aspirin, ibuprofen, and non-steroidal anti-inflammatories, nicotine or other transdermal delivery patches, diet pills or other medications or dietary supplements which may have interfered with test evaluations.
9. Volunteers had an erythemic response to a topical application of (10ul, 2.5 mM) methyl nicotinate.
10. Volunteers had a current (within a year) physical exam.

Exclusion Criteria – Volunteers were not allowed to participate as test subjects if any of the following existed:

1. Female volunteer was pregnant or breast-feeding.
2. Volunteer had a skin disorder or condition that would interfere with test evaluations (e.g., eczema, psoriasis, atopic dermatitis, sunburn, significant tanning).
3. Volunteer had a history of chronic or systemic disease including rheumatoid arthritis or other inflammatory disorders, diabetes, high blood pressure, history of epilepsy, severe asthma, or any medical condition that might interfere with cutaneous vasodilation or inflammation.
4. Volunteer was using medication on a regular basis such as antihistamines, insulin, anti-inflammatory agents including corticosteroids, cortisone containing preparations, aspirin, ibuprofen, and non-steroidal anti-inflammatories, nicotine or other transdermal delivery patches, diet pills or other medications or dietary supplements which may have interfered with test evaluations.
5. Volunteer had a known allergy or sensitivity to one or more components of test materials including adhesives and latex.
6. Volunteer was a smoker.
7. Volunteer was a moderate or heavy drinker who would not likely be able to refrain from alcohol consumption for 24 hours prior to testing.
8. Volunteer did not have a normal reaction to topical application of methyl nicotinate.
9. Volunteer reacted to skin application of SERPACWA.

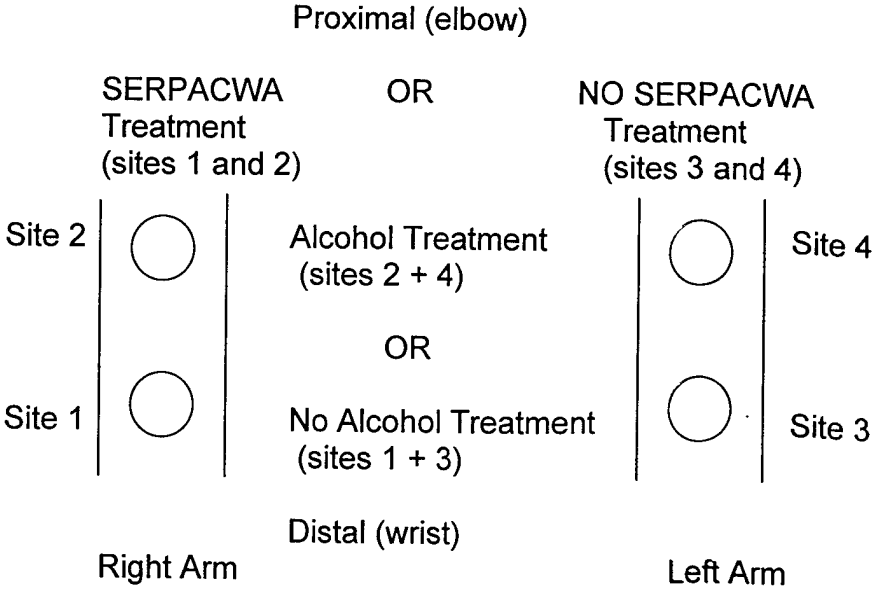
During this clearance process, to test the volunteers for any unusual reaction to SERPACWA, one of the study investigators trained in SERPACWA application (per study SOP) applied 50 ul of SERPACWA to a 2.4 cm diameter site on the volar surface of each volunteer's forearm and removed it after 10 min. To test the volunteers for reactivity to Mnic, 10 ul of 2.5 mM Mnic was applied to another site on each volunteer's

forearm and was removed in 2 min. Both investigator and physician observed the skin reactions to these products at 15, 30 and 60 min post application. Each volunteer was admitted to the study as a test subject when the physician cleared him, and when he met/did-not-meet the inclusion/exclusion criteria.

Forearm Treatment Sites

Prior to testing, a black felt template was made for each subject and was used as a stencil for defining and marking the test sites on the volar surface of the forearms near the wrist. Four 2.4 cm circular test sites (two on each forearm, spaced approximately 1 cm apart), free of scars, blemishes or tattoos, were identified with a skin-safe marking pen. The template was designed so that the test sites' distal boundary began about 2 cm proximal to the wrist crease. The template was also placed on the forearms prior to initiating each LDI scan to provide contrast for LDI flux graphic display. Each site on a SERPACWA-treated arm had a control (no SERPACWA treatment) site at the same relative position on the contra lateral forearm. The arm selected for SERPACWA treatment (right or left) and the sites selected for alcohol pre-treatment (proximal or distal) were randomly assigned. For our sample of six subjects, two received SERPACWA treatment on the right arm and four received it on the left arm. The alcohol pre-treatments were on the proximal forearm sites for three subjects, and on the distal forearm sites for the other three subjects. Figure 1 illustrates the forearm treatment sites.

Figure 1. This diagram illustrates the configuration of the four treatment sites on the volar surfaces of the subjects' forearms.



SERPACWA application on left or right arm, and alcohol treatment on proximal or distal sites were randomly assigned for each of the six subjects. Mnic was applied to all

four sites. The randomized application/treatment sites for each of the six subjects were as follows: Left/Proximal, Left/Distal, Right/Distal, Left/Proximal, Left/Distal, and Right/Proximal.

SERPACWA Application

A fixed volume, positive-displacement micro-dispenser was used to deliver 50 ul of SERPACWA to the center of the test sites for a dose of 11 ul/cm^2 of skin area. The Sponsor specified this dose to maintain consistency with prior SERPACWA efficacy testing on animals and humans (7). This dosage allows for a 10% loss due to potential wastage during application; the net dose being approximately 10 ul/cm^2. A Teflon®-coated spatula was used to spread the SERPACWA evenly over the area for a thickness of approximately 0.1 mm. After the last LDI scan, the SERPACWA was removed with a small, flat, dull blade, Teflon®-coated spatula. The subjects' arms were then washed with warm water and soap to remove any remaining SERPACWA.

Methyl Nicotinate Preparation and Application

Methyl nicotinate (≥99% purity, Sigma Aldrich Chemicals) was used as the challenge agent in this study. Methyl nicotinate (methyl 3-pyridinecarboxylate), a lipid soluble ester of nicotinic acid, is a well-studied contact irritant, producing easily monitored reactions. The non-immunologic contact reaction is due to increased prostaglandin, an inflammatory mediator released after Mnic penetration through the stratum corneum into the dermis (4, 5, 14, 18, 25, 28, 32). The exact dosage and timing of the Mnic challenge used in this study, 10 ul of 5 mM Mnic for 2 min, were determined in the first experiment conducted under this study protocol (16). That experiment was conducted to determine the best dose and timing of Mnic to be used for the remaining experiments. A 2-min exposure of 5 mM Mnic resulted in an erythema that was visible in less than 10 min and increased in intensity to peak at 12-22 min post challenge, and gradually decreased thereafter (16). The majority of healthy adults have this response to Mnic (the inclusion criteria for volunteer clearance in this study was a visible reaction to 2.5 mM), which is otherwise innocuous at the dilute concentrations and limited duration of exposure employed in this study. The Mnic stock solution (50 mM in distilled water) was prepared from the crystalline solid each test day. The 5 mM test solution was also prepared from the stock solution each test day. At 60 min post-SERPACWA application, a 10 ul volume of the 5 mM Mnic solution was applied to each of the four test sites. Mnic was removed 2 min after application by use of a cotton swab to wick the droplets off each test site, followed by blotting with a cotton gauze square (2 x 2 in).

Scanning Laser Doppler Imagery

Flux measurements by laser Doppler imaging (LDI) and visual scoring have been routinely used to assess erythema (7, 9, 19, 30). LDI provides a sensitive, accurate, reproducible and noninvasive means of measuring changes in skin blood flow (10, 11, 14, 18, 19, 25). The LDI scanning technique used in this study (Moor Industries, Inc. Scanning LDI Unit) provides a 2-dimensional pattern of microcirculation, produces a visual image and quantification of perfusion intensity and area. The LDI scans were used to determine the skin response to Mnic, assess the efficacy of SERPACWA, and assess the effect of the alcohol pre-treatment. This LDI technique provides less variance than single point technology, and has the ability to evaluate several test sites in a single scan (24). The technique has been validated by numerous studies and has been found to be highly reliable in assessing changes in blood flow resulting from cutaneous exposure to Mnic (2, 6, 13, 17, 19, 22-24, 27, 32). In addition, the changes in blood flow reported by LDI have been well correlated with erythema; the LDI data can be corroborated with visual scoring (2, 18), as was performed in this study. The validity and reliability of LDI have also been demonstrated in the Phase 3 clinical investigation "The Protective Efficacy of the Topical Skin Protectant (TSP) Against Methyl Nicotinate Under Sweating Conditions" (7). That investigation was sponsored by the Army (USAMMDA) and was presented as one of two pivotal studies in support of the New Drug Application submitted to and approved by the FDA.

For each scan, subjects placed their forearms in a custom made rest that positioned their hands in supination with the forearms and wrists close together, directing the volar test surfaces upward, toward the laser. Subjects wore laser eye protective glasses during the scans. All four sites were scanned for baseline measurements before treatment.

Visual Evaluation

The visual evaluation was used as a secondary assessment of skin reaction to Mnic and was used to corroborate the primary endpoint from the LDI flux measurements. Visual evaluation has been shown to correlate with LDI flux data when exposure to Mnic was assessed (2, 19). The technique originally used a 5-point scale from 0 to 4 and was first reported as a means to assess skin lesions (9). Since its introduction and modification to include half integers, visual evaluations have been widely used as a clinical and research tool (2, 12). In addition, visual evaluation is a FDA requirement in Phase 1 studies for all topically active IND pharmaceuticals and biologics under clinical investigation. Visual evaluations were also used in the two clinical studies pivotal to the FDA approval of the NDA for SERPACWA: the "Sweat Study" (7) and the "Poison Ivy Studies" (29, 30). Visual scores in the "Sweat Study", used to corroborate LDI flux data, correlated with all levels of erythema from Mnic exposure (7). In the current study the visual evaluations of all test sites were done prior to alcohol or SERPACWA application (baseline), and following the LDI flux measurements after the Mnic challenge. An experienced evaluator used a 7-point scale

from 0 to 3 (7) with intermediate scores of 0.5 used at the evaluator's discretion, defined as follows:

0 = no reaction, no erythema
1 = mild reaction, minimal macular erythema, faint but definitely pink
2 = moderate reaction, moderate macular erythema, definite redness, possible edema
3 = strong to severe reaction, intense redness, definite edema, possible spreading

STATISTICAL ANALYSES

Three sets of paired t-tests were performed. (1) To determine if cleaning the skin with isopropyl alcohol prior to SERPACWA application is necessary for its effectiveness against Mnic, LDI flux and visual scoring after Mnic challenge (scan 3), the no alcohol + SERPACWA treated site was compared to the alcohol + SERPACWA treated site. (2) To determine that alcohol itself had no effect, LDI flux before alcohol treatment (scan 1) was compared to alcohol treatment alone (scan 2, site with no SERPACWA). (3) To determine the effect of alcohol in the absence of SERPACWA, LDI flux and visual scoring after the Mnic challenge (scan 3) on the alcohol pre-treated site (no SERPECWA) was compared to the no alcohol pre-treatment site (no SERPACWA). The Mnic-only site served as a test of validity that the skin responded to the Mnic challenge. As reported in the Test Subject Selection section, all volunteers were screened for Mnic responsivity; only responders were included in further testing. For all comparisons, significance was accepted at the 95% confidence level ($p<0.05$).

RESULTS

Figures 2 and 3 illustrate the mean (±SD) flux and visual score data for the four forearm test site conditions. As can be seen in the first two pairs of bars in each graph, there were no differences ($p>0.05$) in either the flux measurements or the visual scores when comparisons were made between the SERPACWA protected sites that were either pre-treated with alcohol or were not pre-treated. However, data observation led us to conduct an additional comparison to determine the effect of alcohol in the absence of SERPACWA. As is evident in the second two pairs of bars in each graph, LDI flux and visual scoring after the Mnic challenge with no SERPACWA protection on the alcohol pre-treated site were significantly ($p<0.05$) higher than those for the site with no alcohol pre-treatment. To test the effect of alcohol alone, flux measurements from the baseline scan were compared to scan 2 for the site that was treated with alcohol but not with SERPACWA. There was no significant effect of the alcohol treatment alone. Table 1 lists the flux and visual score data for 3 (flux) and 2 (visual score) scans for each of the six subjects.

Figure 2 shows mean (±SD) Flux Measurements from scans at Baseline and after Mnic Challenge for six test subjects. AS = Alcohol + SERPACWA, NAS = No Alcohol + SERPACWA, ANS = Alcohol - No SERPACWA, and NANS = No Alcohol - No SERPACWA. * For the 2 NS sites, Flux measurements for the alcohol pre-treated site (ANS) were significantly greater than those for the site not pre-treated (NANS).

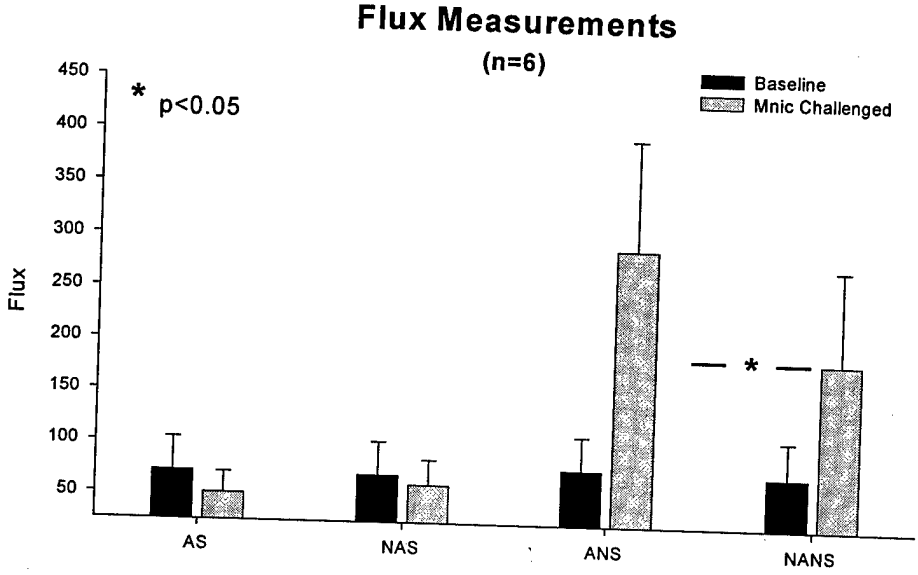


Figure 3 shows mean (±SD) Visual Scores from scans at Baseline and after Mnic Challenge for six test subjects. AS = Alcohol + SERPACWA, NAS = No Alcohol + SERPACWA, ANS = Alcohol - No SERPACWA, and NANS = No Alcohol - No SERPACWA. * For the 2 NS sites, Visual scores for the alcohol pre-treated site (ANS) were significantly greater than those for the site not pre-treated with alcohol (NANS).

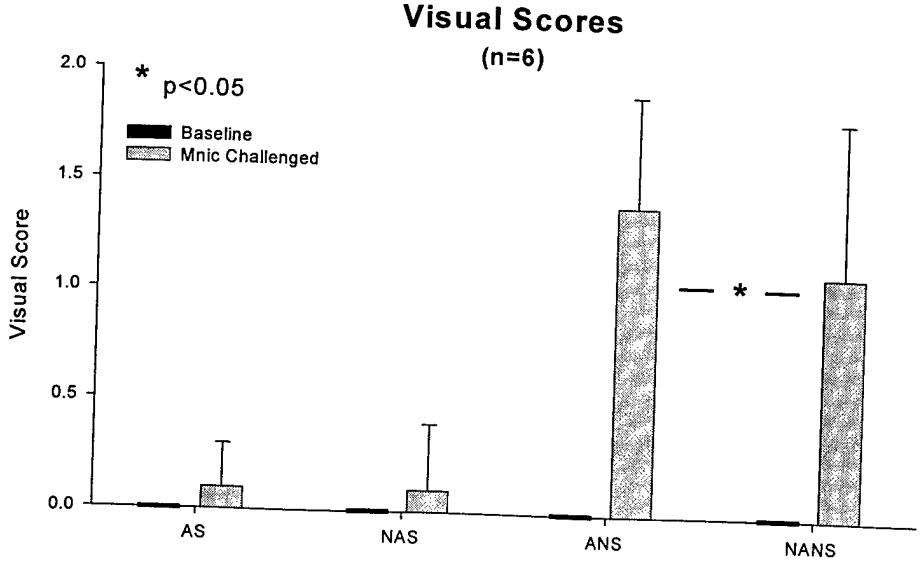


Table 1. LDI Flux and Visual Score Data for each of the 6 test subjects.

SCAN 1: Baseline (no Alcohol, no SERPACWA, no Mnic) FLUX / Visual Score Data				
Subj #	Test site: Alcohol+SERP	Test site: NoAlcohol+SERP	Test site: Alcohol-No SERP	Test site: NoAlcohol-NoSERP
11	52.4 / 0	59.5 / 0	55.7 / 0	57.7 / 0
12	116.1 / 0	121.9 / 0	121.3 / 0	118.4 / 0
14	71.8 / 0	53.2 / 0	89.5 / 0	53.9 / 0
15	40.5 / 0	35.1 / 0	41.1 / 0	44.8 / 0
16	105.0 / 0	97.2 / 0	106.0 / 0	117.7 / 0
17	44.7 / 0	56.0 / 0	54.9 / 0	49.2 / 0
mean	72 / 0	70 / 0	78 / 0	74 / 0
SD	32 / 0	32 / 0	32 / 0	35 / 0

SCAN 2: Post-Alcohol, Post-SERPACWA Application, no Mnic FLUX (no visual scores) Data				
Subj #	Test site: Alcohol+SERP	Test site: NoAlcohol+SERP	Test site: Alcohol-No SERP	Test site: NoAlcohol-NoSERP
11	45.4	56.1	66.7	68.7
12	129.6	151.1	140.2	117.1
14	61.5	41.9	82.1	52.1
15	32.8	41.2	30.7	43.2
16	109.8	106.8	92.6	184.4
17	59.9	44	48.7	52
mean	73	74	77	86
SD	38	45	38	55

SCAN 3: 15 min Post-Mnic Challenge (Mnic on all 4 sites) FLUX / Visual Score Data				
Subj #	Test site: Alcohol+SERP	Test site: NoAlcohol+SERP	Test site: Alcohol-No SERP	Test site: NoAlcohol-NoSERP
11	34.7 / 0.0	38.3 / 0.0	103.9 / 1.0	76.9 / 0.5
12	60.6 / 0.0	67.3 / 0.0	371.7 / 1.5	310.8 / 1.5
14	44.2 / 0.0	36.2 / 0.0	329.0 / 1.0	226.5 / 0.5
15	29.4 / 0.0	62.2 / +	317.6 / 1.0	102.0 / 0.5
16	86.0 / 0.5	102.6 / 0.5	379.6 / 2.0	243.0 / 1.5
17	53.0 / +	57.1 / +	244.1 / 2.0	149.4 / 2.0
mean	51 / 0.1	61 / 0.1	291 * / 1.4 *	185 / 1.1
SD	20 / 0.2	24 / 0.3	104 / 0.5	90 / 0.7

* Alcohol – No SERPACWA sites had significantly greater FLUX and Visual Scores compared to No Alcohol – No SERPACWA sites ($p<0.05$).

+ According to the scorer, the visual score was greater than zero, but less than scoreable (zero was used in the statistical evaluation).

DISCUSSION

After analyzing the results of this experiment, we were able to answer the initial question of whether or not cleaning the skin with isopropyl alcohol before SERPACWA application was necessary. These results show that not only is alcohol pre-treatment not necessary, but that in the absence of the protective SERPACWA, its use may exacerbate the deleterious skin reaction effect of a challenge agent.

One explanation for this exacerbating effect of alcohol pre-treatment may be due to its removal of naturally occurring skin oils of the stratum corneum. These surface fats and oils are part of the skin's natural barrier, defense against water loss from the body and defense against entrance of substances from the environment (26). Isopropyl alcohol is a neutral solvent whose chemical characteristics allow miscibility with water as well as with many oils, both essential (those found on the skin) and other oils and for cellulose derivatives (26). Alcohol sponging, along with insect bites, abrasion or the introduction of a foreign body are all reported as plausible causes of disruption of the intact integument (31). Isopropyl alcohol is listed as a marginal irritant (1).

After conducting this experiment and after noting the results, the remaining experiments in the study did not include alcohol pre-treatment.

CONCLUSIONS

Two noteworthy conclusions can be made based on this experiment. (1) Isopropyl alcohol pre-treatment does not affect SERPACWA's efficacy. (2) When SERPACWA is not used as a skin protectant, isopropyl alcohol pre-treatment exacerbates the skin reaction effects of the challenge agent Mnic.

RECOMMENDATIONS

Naturally occurring oils on the surface of the skin serve to protect against loss of body water and against entrance of foreign substances. These oils may, in small part, work in conjunction with SERPACWA to protect the skin. Since isopropyl alcohol removes these natural skin oils and has no affect on SERPACWA's effectiveness, it is recommended that the practice of cleansing the skin with isopropyl alcohol prior to SERPACWA application be discontinued. In addition, it is recommended that skin products containing a large percentage of isopropyl alcohol not be used prior to SERPACWA application and not be used prior to entrance into a chemical warfare agent threatened environment whether or not SERPACWA is used.

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